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Progress Report

RESISTANCE OF PINES TO BARK BEETLES

Studies on Toxicity of Resins

and

Response of Beetles

1957

By R. H. Smith

NOT FOR PUBLICATION

PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION
FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE

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Progress Report

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Studies on Toxicity of Resins and Response of Beetles

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SUMMARY

This report covers the 1957 season's work on the susceptibility or resistance of pines to bark beetles. The major effort, as in 1956, was devoted to research on fumigant toxicity of pine resins to adult beetles, though an increasing amount of attention was given to studies of contact toxicity and of response by beetles to resin and other pine materials.

The procedures developed in 1956 were used with certain refinements. All resin samples were weighed so that dosage could be based on the weight of the volatile material in the fumigation chamber rather than on the volume of resin used. Except where noted the test beetles were always adults of _Dendroctonus brevicomis_ Lec.

Neoprene was tried in an effort to improve on the cork stoppers to close the fumigation chamber. Several tests were conducted before it was found that neoprene was even less suitable than cork. Comparisons were made between cork, polyethylene, and neoprene. Cork was found to be most suitable. Neoprene was found to be completely unsuitable and, therefore, all tests with it were discarded. Cork was used as the stoppering material, even though it absorbed the resinous vapors to a certain degree.

Tests were also conducted to compare some of the physical properties of resins, which could be responsible for fumigant action. These tests were not fully satisfactory because of the limitations imposed by the use of cork. In general they showed that ponderosa pine resin has the highest percent volatility, while Jeffrey pine resin has the highest vapor saturation. The Jeffrey x ponderosa hybrid is intermediate between the two parents in both respects.

Fumigant toxicity tests, in which the resinous vapors were confined, revealed little difference in the toxicity of vapors which came from resin at different times. That is, the first hour's vapors from a given sample of resin were about the same in apparent toxicity as those which came from it several hours or days afterwards. The toxicity of ponderosa resin did not increase with time after the first 24 hours exposure of the beetle to it. On the other hand the toxicity of Jeffrey pine resin vapors increased quite markedly with increased time of exposure.

Fairly equal amounts (by weight) of vapor from ponderosa and Jeffrey pine resin produced mortality rates that were quite similar.

Tests to compare the effect of the vapors of ponderosa, Jeffrey, and Jeffrey x ponderosa pine resin on D. brevicomis and Dendroctonus jeffreyi Hopk. were not fully satisfactory. However, the reaction of the beetles to the artificial conditions of the tests seemed to be parallel to what might be expected from field observations on the host relationships of these two insects. D. brevicomis tolerated ponderosa resin vapors much better than it tolerated Jeffrey resin vapors while D. jeffreyi tolerated Jeffrey resin vapors much better than it tolerated ponderosa resin vapors.

Comparisons were made between fresh resin of ponderosa pine and two of its derivatives: turpentine obtained by commercial fractionation and supernatant liquid obtained by natural crystallization. In a saturated atmosphere there was little difference in the toxicity to D. brevicomis of vapors of fresh resin and the supernatant liquid. But there was a distinct difference between these two materials and turpentine, due perhaps to a change in composition of turpentine by heat fractionation.

Humidity appears to be an important factor in fumigant toxicity as obtained by the use of the 30 cc. test tube technique. In general, increased humidity completely nullified the fumigant toxicity of ponderosa resin. Increased humidity always increased the toxicity of Jeffrey pine resin to D. brevicomis. Increased humidity also appreciably decreased the rate of natural mortality though it did not increase the maximum length of life of non-feeding beetles.

Fumigant toxicity tests in which the resinous vapors were restricted but not confined (therefore called semi-confined), produced different results than confined vapors. When semi-confined, Jeffrey pine resin had practically no effect on D. brevicomis, while ponderosa resin still caused a significant increase in mortality rate.

In studies of contact toxicity there was little difference in the mortality rate of D. brevicomis given a single complete coating of fresh resin of ponderosa, Jeffrey, or Jeffrey x ponderosa pine. The mortality rate of treated beetles increased noticeably in comparison to ones which were not treated. The single coating of ponderosa resin appeared to increase the ability or inclination of the beetles to feed on ponderosa bark.

A Y-type olfactometer was constructed for studies of response of adult beetles. Tests with *Dendroctonus valens* Lec. showed an equal choice for either arm on the first run, but beetles which were run a second time showed a decided preference for the direction of their initial choice. This species also showed a decided preference for the arm containing a piece of pitch tube. *D. brevicomis* showed the same equal selection for the two arms and also the same tendency to select the arm of its original choice. However, this beetle showed no preference for various stimuli.

INTRODUCTION

This report represents the second season's work on toxicity and response as part of the project of "susceptibility or resistance of pines to bark beetles." The introduction to the 1956 report [1/] covers the general objectives of the work and specifically the reasons for studying the toxicity of pine resins to bark beetles. Briefly, Painter[2/] classifies the factors responsible for plant-resistance to insects into three main categories: non-preference, antibiosis, and tolerance. He also states that plant-resistance research should be concerned with the plant tissues and materials which are intimately associated with the insect. The background of literature on pine bark beetles implies a very close relationship between resin and the beetle. Callaham[3/] suggests that resin may be toxic through quality or quantity. Thus the decision was to pursue studies of the toxicity of fresh pine resin to bark beetles.

As in the 1956 studies, the major effort was devoted to an investigation of the fumigant toxicity of pine resins to bark beetles. However, there was less total time spent on fumigant toxicity so that more time could be used for investigating the questions of contact toxicity and bark beetle response. Thus the major portion of the report concerns fumigant toxicity, while there are two short sections on contact toxicity and bark beetle response.

To a large extent the course of the work reported here was determined by the results of the 1956 work. In general an effort was made to expand and to refine the more promising leads which developed from that work. This indicated that adults of western pine beetle, Dendroctonus brevicomis Lec. were able to tolerate a saturated resin vapor atmosphere of (1) Jeffrey pine for just a little more than a day, (2) ponderosa pine for extended periods, though not as well as they could tolerate a non-resinous atmosphere, and (3) the Jeffrey x ponderosa hybrid for an intermediate time between the two parents. It was also evident that these three resins varied greatly in their vapor saturation point and that this could be the cause of their differential toxicity to the

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1/ Smith, R.H. 1959. Progress report, resistance of pines to bark beetles, studies on toxicity, 1956. Calif. Forest & Range Expt. Sta. 43 pp., illus.

2/ Painter, R.H. 1951. Insect resistance in crop plants, 520 pp., illus. New York.

3/ Callaham, R.Z. 1953. Studies of the resistance of pines to bark beetles, season 1952. B.E. & P.Q., Forest Insect Lab. Berkeley, Calif. 27 pp., illus.

beetle. Therefore much of the work was planned to be directed toward a comparison of equal weight and percent saturation of the three resinous vapors.

In 1956 the stoppering of the fumigation chambers presented certain problems which had to be reckoned with in 1957.

The study of beetle response was undertaken to a limited extent during the season's work. Insect response or attraction may be more fundamental to the problem of resistance than toxicity. If a species of tree or if certain individuals of a given species are non-attractive to a bark beetle, they are resistant. A brief background of bark beetle response will be given at the beginning of the section of this report which deals with that topic.

The season's work was carried out at the Institute of Forest Genetics whose facilities and, upon a few occasions, personnel were made available to the work. Mr. Stanley Krugman, a graduate student in plant (tree) physiology at the University of California assisted in the collection of the data, and Mr. Richard Krebill, a University student, assisted in the examination of some of the material.

GENERAL PROCEDURES

The general procedures for obtaining resin, for rearing beetles, and for conducting the various tests, especially those on fumigant toxicity, were essentially those used in 1956. To avoid repetition these are briefly described here.

Resin

The resin was extracted from the tree by first making a punch wound, $1\frac{1}{2}$ inches in diameter, through the bark and $\frac{1}{4}$ to $\frac{1}{2}$ inch into the sapwood (figure 1). A plastic cylinder was pushed into the hole and the resin collected in a 30 cc. shell vial which was attached to a hole in the lower surface of the cylinder. In all tests the same three trees were used. One was a ponderosa pine (*Pinus ponderosa* Laws), one was Jeffrey pine (*P. jeffreyi* Grev. & Balf.), and one was a hybrid of the two (*P. jeffreyi* x *ponderosa*).

Beetle

Several different brood trees were used during the season. All were located in the vicinity of the Institute of Forest Genetics or the Eldorado National Forest.

For *D. brevicomis*, the infested bark was removed from either a felled or standing ponderosa pine (figure 2). The brood in the bark was usually in the late larval stage at the time the bark was removed. The bark was stored in a cold room at the Institute at 35°F. until

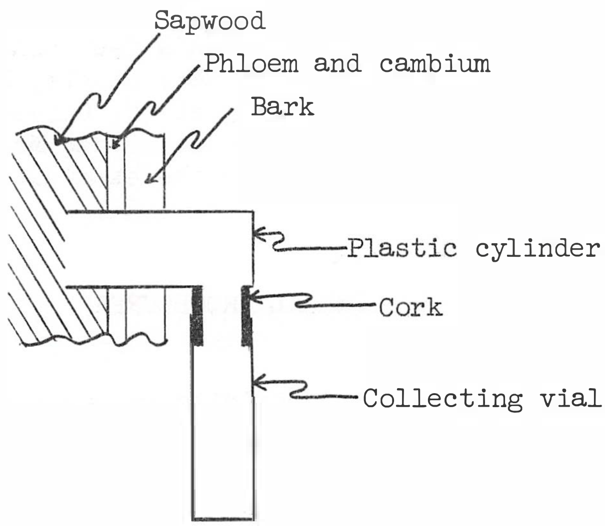

Figure 1.--Diagrammatic cross section of part of tree and resin-collecting device.

needed. It was then placed in one of the 8 units of the insectary (figure 3) which had been built early in 1957. By putting a new supply of bark in a different unit every 2 weeks, it was possible to have beetles at all times. For Dendroctonus jeffreyi Hopk. an infested Jeffrey pine, about 28 inches d.b.h., was cut and sectioned early in the summer when the brood was in the late larval and pupal stages. Short bolts from this tree were handled like the bark of ponderosa pine. Thus, for D. brevicomis only the infested bark was needed since the brood matures in the bark, but for D. jeffreyi it was necessary to use the entire bolts of the tree since the brood matures between the bark and the wood.

The beetles, when they emerged in the insectary, were collected individually in gelatin capsules, usually twice daily, and were held at 35°F. until used in tests. Beetles held in cold storage more than 48 hours were not used.

High midday temperatures in the insectary units proved to be somewhat of a problem. To depress the high temperatures, side "curtains" of coarse burlap were hung along the edges of the roof. A water sprinkler on the roof kept the burlap wet. Cooling was accomplished by natural evaporation. With this procedure midday temperatures were lowered 3 to 5°F.

Just before use, all beetles were equitably distributed by size and age among all the replicates of each test.

Assembly of tests and recording of data

Many of the fumigant tests were assembled like those described in the 1956 report. Such tests will be called "standard." Deviations from the standard will be properly noted.

The actual assembly was as follows (figure 4).

1. A replicate of beetles was taken from refrigeration. (The refrigerated beetles were inactive for a minute or two and, therefore, were easy to handle.) Each beetle was transferred from a gelatin capsule into a fumigation cell, four such cells being bundled together.

2. Two or three of these bundles were placed in the fumigation chamber and a disk of 32-mesh lumite plastic screening was placed on top of the bundle.

3. A glass cylinder cap was placed on top of the plastic mesh disk until it was replaced by a resin vial.

4. Resin was apportioned into a resin vial with a pipette and was then placed in the fumigation chamber. The resin vial was weighed with an analytical balance at designated times in order to obtain the loss of weight of the resin. This was assumed to be the weight of the volatile material in the chamber.

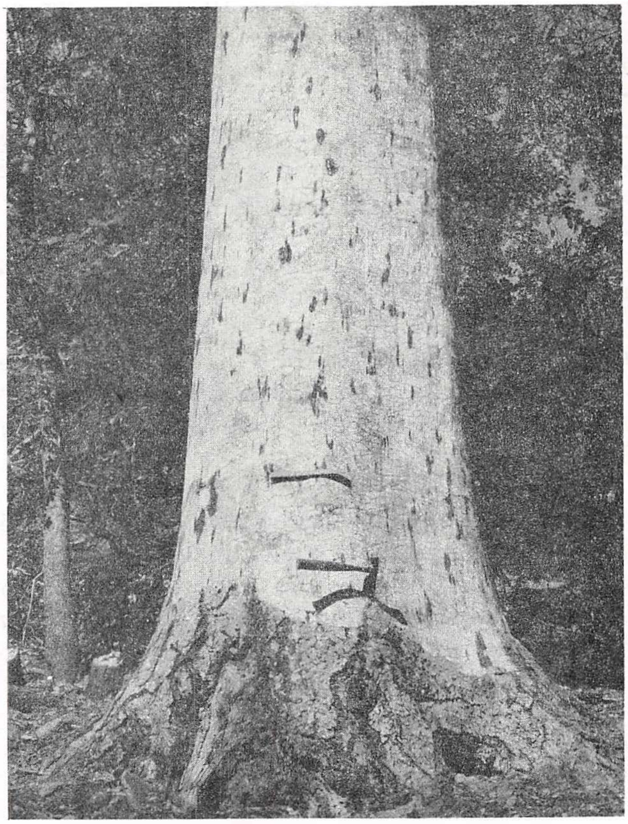

Figure 2.--Ponderosa pine from which D. brevicomis brood bark was removed.

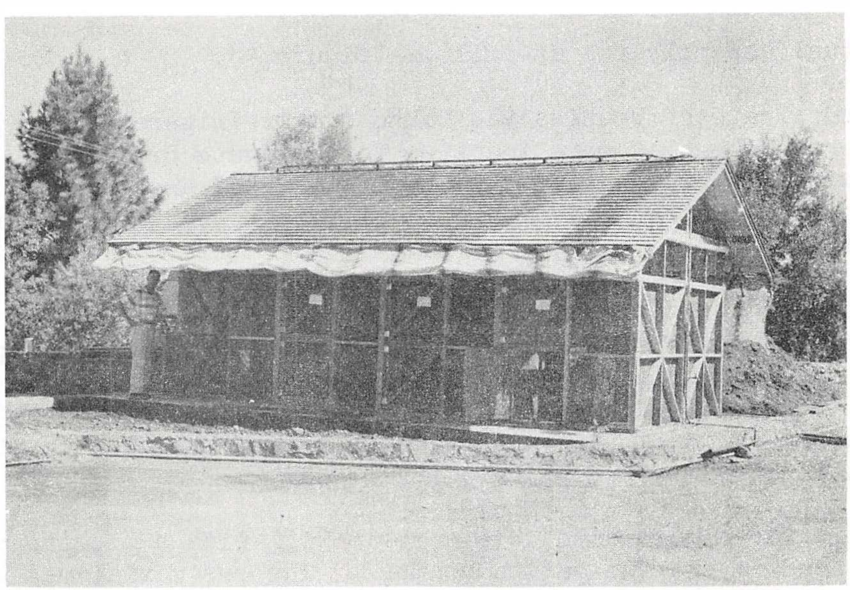

Figure 3.--Insectary at the Institute of Forest Genetics with 8 individual cubicles each about 6 ft. x 6 ft. x 6 ft.

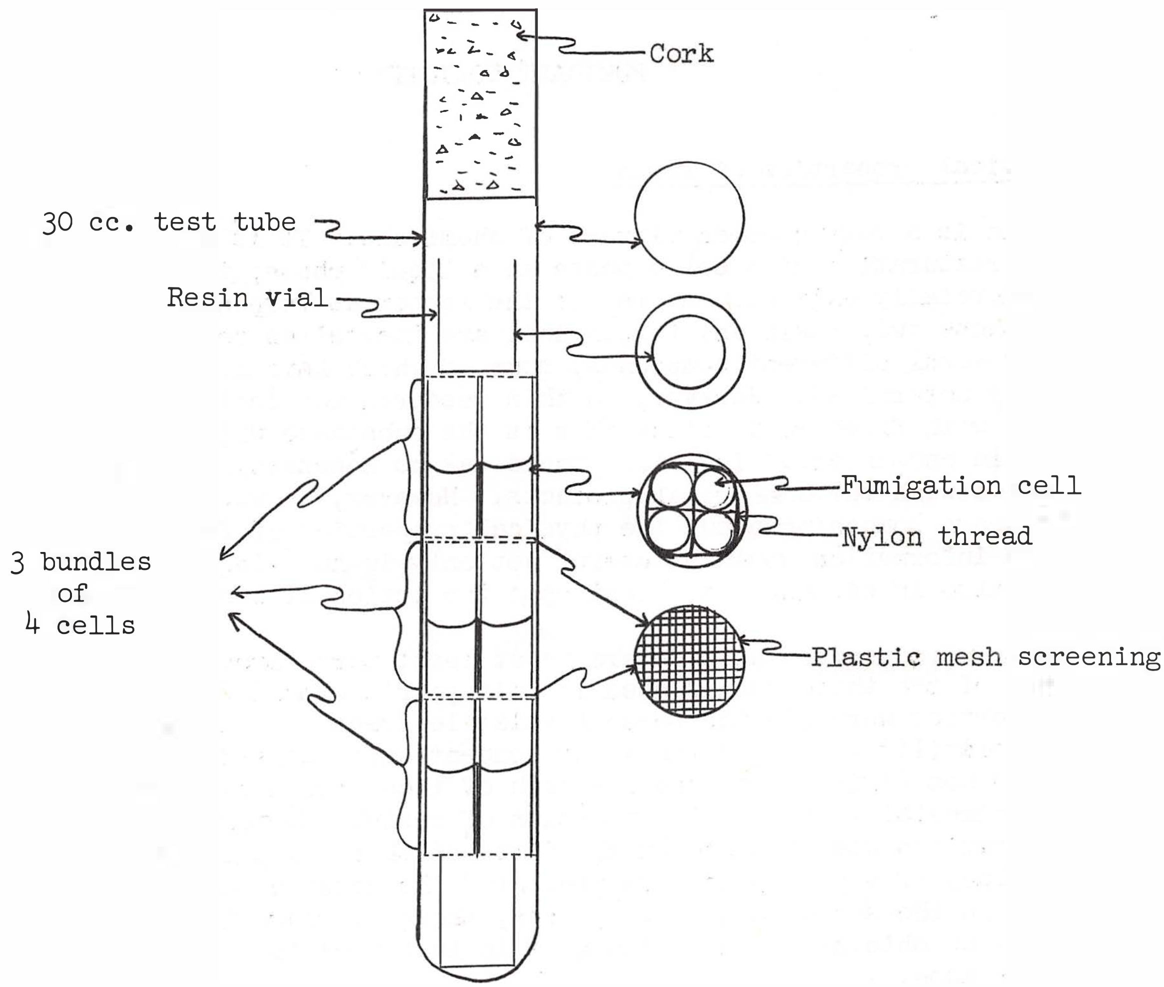

Figure 4.--Longitudinal and cross-sectional diagrams of fumigation chamber.

5. The weighed resin vial was placed on top of the last disk of screening.

6. The assembled chamber was placed under the conditions of the test.

7. All tests were maintained at 70°F. ± 2°. Examinations were made daily and beetles were considered dead which did not move when agitated. Sex determinations were made post mortem.

FUMIGANT TOXICITY

Physical properties of resin

Resin is a very complex mixture of chemicals. It is basically a supersaturation of a solid phase in a liquid phase; the former is commercially extracted as rosin; the latter as turpentine. Each of these two, rosin and turpentine, are themselves complex mixtures of several different compounds, some of which have not yet been fully determined. However, in this research the decision was to work with fresh resin since this is the substance which a bark beetle encounters. Therefore there was no necessity to be concerned with the commercial products. However, it was important to learn something about the physical properties of fresh resin. Such information would be useful not only in handling the resin but also in casting some light upon the action of resin on beetles.

Tests were conducted to determine at least three properties of each of the three resins used in this year's experiments. These properties were (1) the percent volatile fraction, (2) the rate of volatility, and (3) the vapor concentration at saturation. It is not difficult to see how each of these could be quite instrumental in the fumigant action of resin. Reasonably sound information was obtained on the first of these properties. But the lack of a good procedure prevented the securing of reliable data on the second and third points, though a rough idea about them was obtained from handling resin in the various tests during the season.

Because of the uncertain action of cork, data on vapor saturation from these tests must be given with much reservation. However some idea of vapor saturation may be gained by comparing the results of various tests with the three resins under the same conditions. In these tests, each run at 70°F., a 30 cc. test tube with a cork stopper and an excess of resin were used. The average amount of vapor required to produce saturation at the end of a 12-hour period for each of the tree resins was:

ponderosa	--- 1.5	milligrams
Jeffrey x ponderosa	--- 5.0	"
Jeffrey	--- 8.0	"

If it is assumed that cork absorbs all three resins somewhat proportionately, it appears that Jeffrey pine resin has a vapor saturation of about five times that of ponderosa pine resin. The hybrid is intermediate between the two parent trees. This relationship held true in all tests.

Very little can be said about the rate of volatility. Several factors influence this property and no realistic data were obtained. However, based on the handling of the various resins under various conditions during the course of the season's work, it would seem the Jeffrey pine resin has a much greater rate of volatility than ponderosa pine resin.

Fairly reliable data were obtained on the percent volatile fraction of the three resins. There was some variability in the data from time to time probably because of the differences in handling the resin. At 70°F. the following average figures were obtained for the percent volatile fraction:

ponderosa	--- 20-22 percent
Jeffrey	--- 12-15 "
Jeffrey x ponderosa	--- 13-16 "

Since the data were obtained from the loss of weight of gross fresh resin, one possible error in these figures is weight increase through oxidation. The resin was allowed to vaporize slowly for several days until there was no additional loss of weight. However weights after this sometimes showed a slight increase. Thus oxidation during the period of vaporization may have offset some of the weight loss.

There is much more which should be learned about these resins. However such information will have to be gathered when it is needed.

Stoppering materials

A. Neoprene vs. cork

As a result of the last tests made in 1956, it was evident that the stoppering of the fumigation chamber and the absorption of resin by the stoppering material were important problems. No difficulties were experienced in tests with saturated atmospheres and excess resin. But in tests where the atmosphere was not saturated the unsatisfactory nature of the stoppering material being used became apparent. With subsaturation conditions the stoppering material must completely close the fumigation chamber but must not absorb the resin vapor. Since cork had been found to absorb the volatile fraction of resin, a change was made to neoprene which was thought to be a nonporous, nonreactive material.

At the outset it was erroneously assumed that neoprene was non-absorptive. Several tests were carried out to determine the rate

of volatility and vapor saturation of the resin of ponderosa and Jeffrey pine and of the hybrid of the two. The faulty data which were secured were then used to carry out tests of toxicity to D. brevicomis by the resinous vapors of these resins at presumably various subsaturation concentrations. As soon as the mortality rate was recorded, it became evident that there was a fault in the technique. This quickly led to a test of the comparative absorption of resinous vapors by neoprene and cork.

The test was designed to compare the toxicity of ponderosa and Jeffrey pine resin vapors to D. brevicomis in a fumigant chamber stoppered with cork or neoprene. The loss of weight of resin in the closed atmosphere was obtained by weighing the resin sample just before it was placed in the test tube and again at the conclusion of the test.

At the time there was also some concern about the comparability of different sources of the beetle. The importance of this factor was studied by using two different sources of beetles. One source was the overwintering generation while the other was the first or second summer generation. The overwintering generation material had been collected in April when the brood was in the last larval stage and held in cold storage about 4 months until used in August. The summer generation material had been collected in July when the brood was also in the last larval stage. It was stored for only a week or two.

Two types of fumigant chambers were used. One was the standard 30 cc. test tube, while the other was a glass tube of equal dimensions. The test tube was closed with a single stopper while the glass tube was closed with two stoppers. This glass tube was used in anticipation of tests later in the season which would require the use of such a device.

The test was set up by the standard procedure, using 1.0 ml. of ponderosa or Jeffrey resin in each chamber. Mortality counts were made daily. At 14 days the resin was reweighed to determine the amount of resin which had volatilized.

The results are given in table 1.

Discussion: No statistical analysis was made since it is readily apparent from the data that the stoppering material radically affects the mortality rate of the beetle in a resinous atmosphere. The two types of stoppering material also differ in their absorption of the volatile fraction of the two resins. As a rule there is no great difference between summer and winter broods. The differences which do exist could be caused by the size of the beetles and the length of time they were held in cold storage. In general the summer and winter broods responded in a parallel manner, whether they were in a treated or untreated atmosphere.

Table 1.--Resin weight loss and cumulative beetle mortality in fumigation chambers stoppered with different materials

Stopper material	Resin 1/	14-day resin weight loss	Brood source	Beetles used	Days after start of test: 1	2	3	4	5	6	7	8	9
		Milligrams		Number	Number								
			Double stopper										
Neoprene	P	93.6	Summer	40	1	4	7	25	40				
Cork	P	19.5	"	40	0	1	7	23	34	36	38	40	
Neoprene	J	64.1	Summer	40	5	23	35	40					
Cork	J	42.6	"	40	40								
Neoprene	P	85.5	Winter	24	3	5	13	18	23	24			
Cork	P	9.7	"	24	3	3	8	12	19	21	23	24	
Neoprene	J	70.8	Winter	24	3	6	17	22	24				
Cork	J	45.6	"	24	24								
Neoprene	-	-	Summer	40	0	1	4	40					
Cork	-	-	"	40	0	2	2	7	8	13	17	21	29
Neoprene	-	-	Winter	24	0	0	2	8	20	24			
Cork	-	-	"	24	0	0	3	4	8	9	15	19	23
			Single stopper										
Neoprene	P	79.6	Summer	24	0	0	1	7	19	24			
Cork	P	13.4	"	24	0	4	6	9	16	19	22	24	
Neoprene	J	64.6	Summer	24	6	19	24						
Cork	J	22.0	"	24	22	24							
Neoprene	-	-	Summer	24	0	0	1	3	18	22	24		
Cork	-	-	"	24	0	0	0	1	3	5	7	10	13

1/ 1.0 ml. of P(ponderosa pine) or J(Jeffrey pine).

The mortality rate of the beetle in the controls demands some attention. The fact that the mortality rate was so much greater in the neoprene-stoppered untreated atmosphere could mean that neoprene was producing a toxic substance either directly, or indirectly as a reaction to the volatile fraction of resin or the products of the insect's metabolism. The depletion of oxygen or the increase of carbon dioxide are other possibilities but it is doubted whether either takes place rapidly enough to account for the sudden high mortality between the 3rd and 6th day.

From the loss of weight of resin it is apparent that the mortality rate does not follow the resin weight loss. Therefore it was concluded that neoprene was absorbing the resin more rapidly than cork. As a result, the use of neoprene was discontinued.

B. Polyethylene vs. cork

When neither cork nor neopene proved to be fully satisfactory materials for closing the fumigant chamber in tests with subsatruation concentrations, other materials were considered. Among those most readily available and suitable was polyethylene. A test was made to compare cork and polyethylene.

The standard procedure was used with both the 30 cc. test tube and the 30 cc. glass tube. D. brevicomis was the test insect. Half of these tubes were stoppered with regular cork while the others were stoppered with corks wrapped with thin sheets of polyethylene. Resin of ponderosa pine and Jeffrey pine was used at a volumetric dosage of approximately 0.5 ml.. Weighings were made of the resin and the stoppering materials. It was assumed that when the "pick-up" by the cork was substracted from the "loss" by the resin the result would represent the actual weight of resinous vapors in the fumigation chamber. At 24 hours and at 48 hours one replicate of each condition was disassembled so that the weight-loss of the resin and the weight-increase of the stopper could be made at realistic intervals. At these periods the beetles were transferred to a clean atmosphere. Thus the factor of time of exposure was added to the test.

The data obtained from the weighings are given in table 2, while table 3 gives the mortality records for the corresponding samples.

Discussion: In general the polyethylene either allowed the resin to pass through to the cork or actually absorbed the resin. Thus polyethylene was no better than cork for closing the fumigation chamber. The wide variation in weight-increase of cork used to close the untreated chambers could account for the irregularities in the test. There was little residual or carry-over effect of the 24-hour exposure of D. brevicomis to Jeffrey pine resin; beetles alive at that time seemed to have a normal subsequent mortality rate. On the other hand there was a carry-over effect of the 48-hour exposure, though with just a 48-hour exposure most beetles were dead. As a result of this test all plans for subsaturation studies had to be discarded.

Table 2.--Weight loss of ponderosa and Jeffrey pine resin at 24 and 48 hours in 30 cc. fumigation chambers

Stoppering material	Resin	Sample no.	Exposure time	Stopper weight increase	Stopper weight increase above check	Resin weight loss	Calculated volatile material in chamber
			Hours	Milligrams			
Single cork	P	1	24	9.9	4.7	3.2	0
	P	2	48	10.1	3.4	6.3	2.9
	J	3	24	5.5	0.2	8.9	8.7
	J	4	48	9.5	2.0	10.1	8.1
	-	5	24	5.3	-	-	-
	-	6	48	6.7	-	-	-
Single cork with polyethylene	P	7	24	6.2	0.4	4.6	4.2
	P	8	48	13.5	5.2	5.7	0.5
	J	9	24	9.0	3.2	11.4	8.2
	J	10	48	8.1	0	18.0	18.0
	-	11	24	5.8	-	-	-
	-	12	48	8.3	-	-	-
Double cork	P	13	24	14.4	4.7	3.3	0
	P	14	48	20.7	0	4.4	4.4
	J	15	24	12.2	2.5	9.1	6.6
	J	16	48	26.3	5.2	15.4	10.2
	-	17	24	9.7	-	-	-
	-	18	48	21.1	-	-	-
Double cork with polyethylene	P	19	24	11.1	0	6.4	6.4
	P	20	48	24.7	0	8.4	8.4
	J	21	24	12.1	0.4	17.0	16.6
	J	22	48	22.0	0	27.4	27.4
	-	23	24	11.7	-	-	-
	-	24	48	25.8	-	-	-

Table 3.--Cumulative mortality of D. brevicomis associated with calculated vapor concentrations of ponderosa and Jeffrey pine resin

Resin	Sample no.	Calculated vapor in chamber	Exposure	Total beetles used	Days after start of test 1	2	3	4	5	6	7	8	9	10
		Milligrams	Hours	Number	Number									
P	1	0	24	8	0	0	0	1	2	2	5	6	6	6
P	2	2.9	48	8	0	0	0	0	0	2	4	5	6	7
J	3	8.7	24	8	6	6	6	6	6	7	7	7	7	8
J	4	8.1	48	8	6	8								
-	5	-	24	8	0	0	0	1	1	1	3	5	5	6
-	6	-	48	8	0	0	1	1	2	3	4	4	4	5
P	7	4.2	24	8	0	0	0	0	0	0	2	4	5	6
P	8	0.5	48	8	0	0	1	1	1	1	3	5	5	5
J	9	8.2	24	8	4	5	6	6	6	6	6	6	6	6
J	10	18.0	48	8	1	8								
-	11	-	24	8	0	0	0	0	0	1	3	3	5	7
-	12	-	48	8	0	0	0	0	0	2	3	3	4	5
P	13	0	24	8	0	0	0	2	2	2	3	5	6	6
P	14	4.4	48	8	0	0	1	5	6	8				
J	15	6.6	24	8	7	7	7	7	7	8				
J	16	10.2	48	8	8									
-	17	-	24	8	0	1	1	2	3	4	5	5	6	7
-	18	-	48	8	0	0	1	1	2	3	5	6	7	8
P	19	6.4	24	8	0	0	0	1	1	2	5	7	7	8
P	20	8.4	48	8	1	1	1	2	3	3	5	6	6	7
J	21	16.6	24	8	6	6	6	6	6	7	7	7	7	7
J	22	27.4	48	8	6	8								
-	23	-	24	8	0	0	0	0	1	3	4	4	4	5
-	24	-	48	8	0	0	1	1	1	4	5	5	6	8

Cumulative and successive fractions of resins

Despite the shortcomings of cork, and while waiting the outcome of the tests on fumigation chamber closure material, one large test was made to compare the fumigant toxicity of the resins of ponderosa, Jeffrey, and Jeffrey x ponderosa pine. The test was extended to study the effect of cumulative and successive volatile fractions of the resin. To obtain the cumulative fractions, the resin was left in the chamber for increasing periods of time. The successive fractions were obtained by shifting the same series of resin samples to a new series of chambers at the designated times. Thus, in the cumulative series all replicates were started at the same time and then disassembled at increasing intervals. In the successive series a new set of replicates was started at each interval using the same resin samples which had been used in the replicates covering the previous interval.

The standard fumigant testing procedure with the 30 cc. test tube and a cork stopper was followed. In all cases the resin was placed on top of the bundles of beetles so that the resin samples could be removed with a minimum of disturbance to the atmosphere in the fumigant chamber. Thus the beetles were retained in the original resinous atmosphere throughout the test period. All resin samples were approximately 0.5 ml.. However all dosages of vapor were determined by the loss of weight of the resin during the particular period of the test. All transfering and weighing of resin was accomplished as quickly as possible. However there was a slight loss of resinous vapors during the removal of the resin vial from the fumigation chamber. Therefore the actual resin vapor in the chamber was slightly less than the calculated. Likewise, there was some loss of resin through absorption by the cork stoppers.

The following time intervals were used with the respective resins.

Series	Cumulative			Successive		
	Ponderosa	Ponderosa x Jeffrey	Jeffrey	Ponderosa	Ponderosa x Jeffrey	Jeffrey
	Hours total			Hours interval		
1	12	3	1	0-12	0-3	0-1
2	24	6	2	12-24	3-6	1-3
3	36	12	4	24-36	6-12	3-5
4	48	24	8	36-48	12-24	5-9
5	60	48	18	48-60	24-48	9-20
6				60-48		20-44

The data are summarized as follows:

Ponderosa resin	table 4
Jeffrey "	" 5
Jeffrey x ponderosa	" 6

Figures 5 and 6 illustrate aspects of ponderosa resin.

Discussion.--As with other tests of this nature, the uncertain action of cork makes an interpretation of the results somewhat questionable. However a few general remarks may be warranted. With cumulative time, ponderosa pine vapors had no appreciable added fumigant effect after the first 24 hours, though there was additional resin-weight loss after this time. This loss was probably going into, or through, the cork rather than into the fumigant atmosphere. There appears to be very little difference in the fumigant effect of successive fractions of ponderosa pine vapors.

With Jeffrey pine resin an increase in cumulative time, slight as it was, noticeably increased the rate of mortality. The increase in resin weight-loss associated with cumulative time was probably caused by absorption by the cork or by escape from the fumigation chamber. Irregularities in weight-loss and mortality prevent a remark about successive time intervals.

With the Jeffrey x ponderosa resin, an increase in cumulative time may have increased the resin weight-loss and the mortality rate of the beetle. But the pattern is not consistent. Successive volatile fractions are not too different in their effect on the mortality rate.

One of the more interesting comparisons is shown by the mortality data for ponderosa and Jeffrey pine at approximately equal vapor dosages. Near-equal dosages were obtained at 60 hours for ponderosa and at 1 hour with Jeffrey. The resin weight-losses were 4.0 and 5.2 milligrams respectively. The cumulative mortality by days (1 to 10) was as follows:

Ponderosa	0	0	2	5	19	25	34	39	39	40
Jeffrey	0	0	2	5	13	23	30	34	34	35
Check	0	0	1	1	1	3	5	10	12	17

This could indicate that toxicity was more dependent on amount and less dependent on quality of the vapors. Though the time of exposure might be considered here, it should be recalled that the beetles were disturbed as little as possible upon removal of the resin sample. The presence of cork is the big question mark. After one hour's exposure to get the 5.2 milligrams of weight loss of Jeffrey resin in the chamber, the cork stopper may very well have promptly absorbed a good portion of it.

Table 4.--Cumulative mortality of D. brevicomis confined with ponderosa pine resin vapors collected at different time intervals

Time	Resin weight loss[1/]	Total beetles used	Days after start of test										
			3	4	5	6	7	8	9	10	11	12	13
Hours	Milligrams	Number	Number										
----Cumulative periods of time-----													
12	1.6	40	0	0	2	6	11	17	29	32	37	39	39
24	2.7	40	2	6	9	22	25	28	33	36	38	39	39
36	3.2	40	1	3	9	18	26	34	35	37	40		
48	2.7	40	0	5	18	26	32	35	40				
60	4.0	40	2	5	19	25	34	39	39	40			
Untreated	0	40	1	1	1	3	5	10	12	17	22	28	31
----Successive periods of time-----													
0-12	1.8	40	3	7	7	14	17	20	27	34	36	38	38
Untreated	0	40	1	1	1	3	5	10	12	17	22	28	31
12-24	2.0	40	4	8	12	14	22	31	38	38	38	39	40
Untreated	0	40	0	2	3	5	8	15	17	25	27	28	38
24-36	1.8	40	1	3	6	9	13	24	28	33	36	38	38
Untreated	0	40	0	2	5	9	10	14	19	22	26	38	38
26-48	1.5	40	2	3	8	17	19	25	31	35	37	38	38
Untreated	0	40	0	2	8	10	15	15	18	24	29	33	33
48-60	2.1	40	1	4	8	11	17	23	30	30	33	33	39
Untreated	0	40	0	4	5	5	9	12	17	22	26	26	36

1/ Each weight an average of 5 samples.

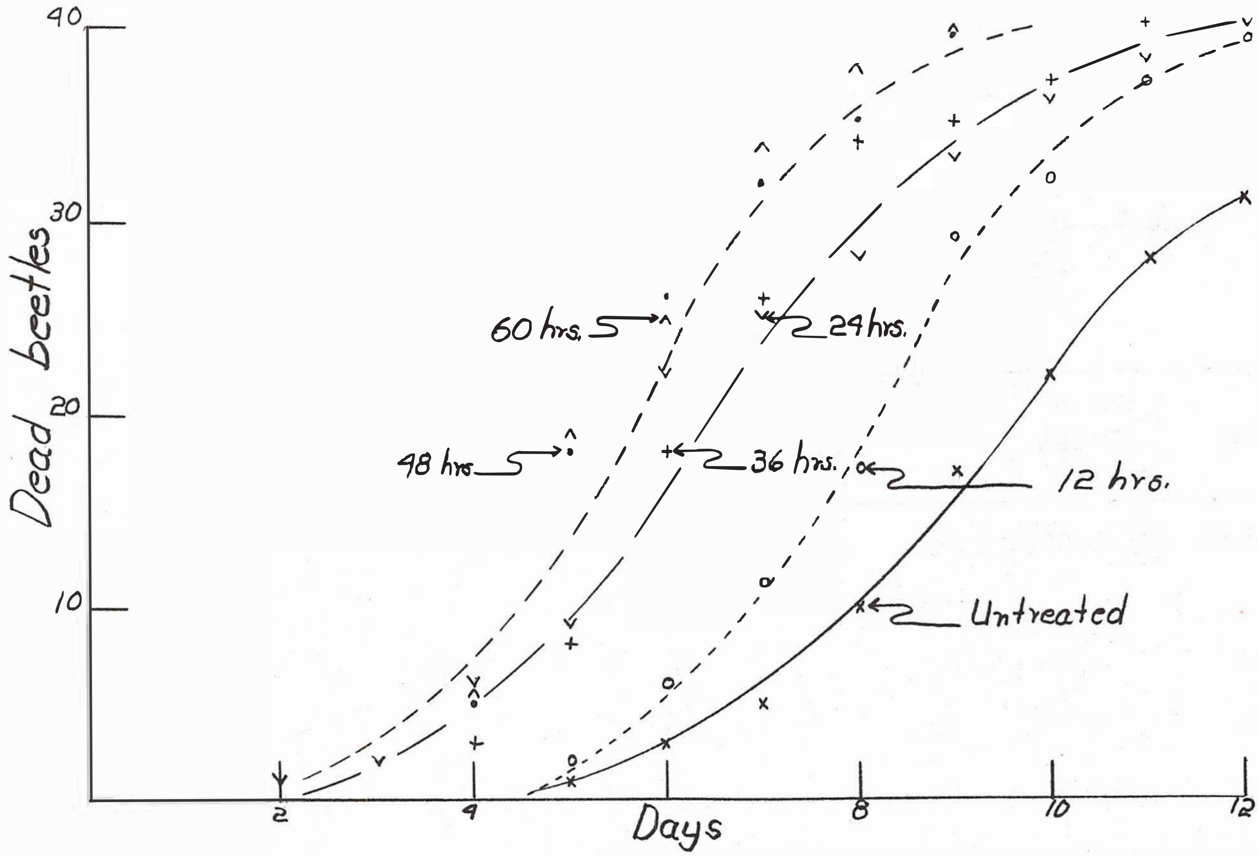

Figure 5.--Mortality rate of D. brevicomis confined with cumulative fractions of ponderosa pine resin vapors.

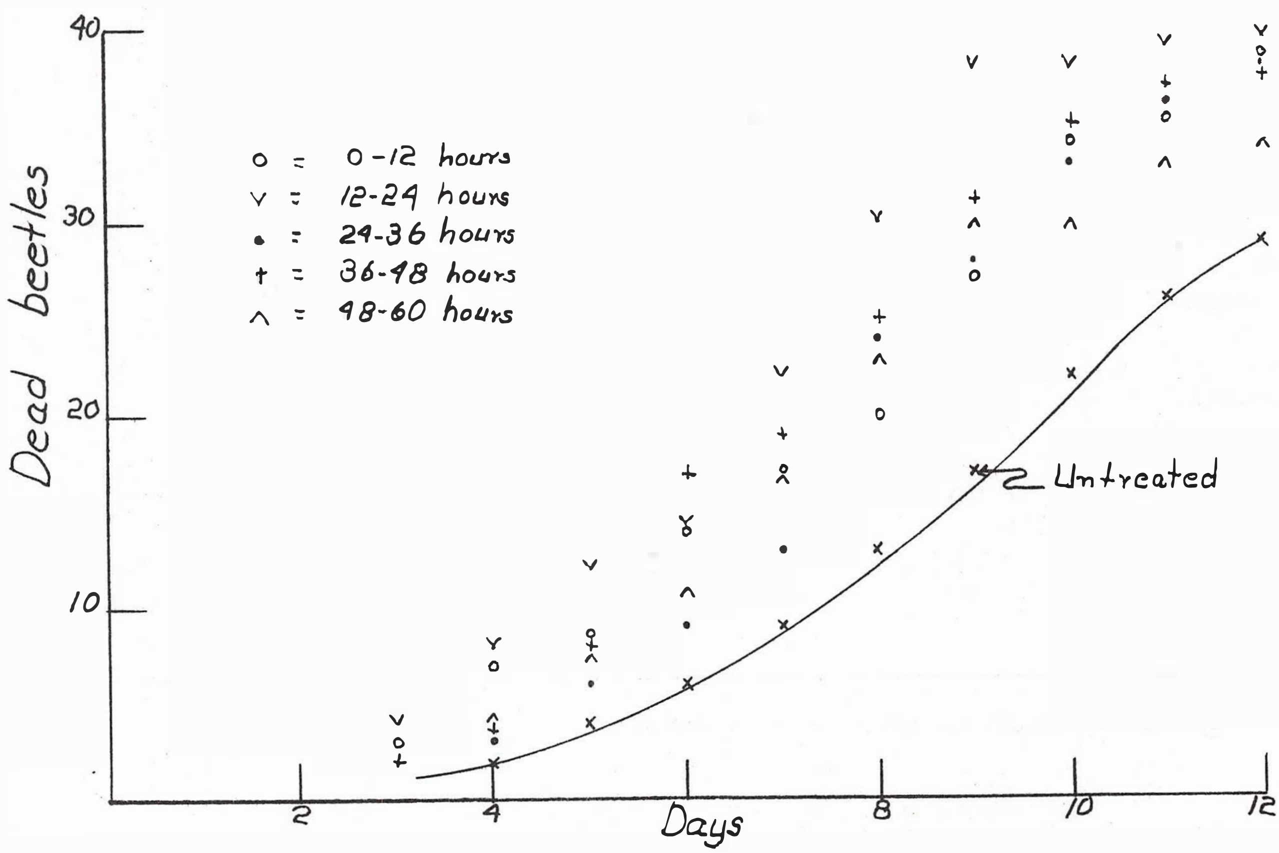

Figure 6.--Mortality rate of D. brevicomis confined with successive fractions of ponderosa pine resin vapors.

Table 5.--Cumulative mortality of *D. brevicomis* confined with Jeffrey pine resin vapors collected at different time intervals

Time	Resin weight loss1/	Total beetles used	Days after start of test 1	2	3	4	5	6	7	8	9	10	11
Hours	Milligrams	Number											
		-----Cumulative periods of time-----											
1	5.2	40	0	0	2	5	13	23	30	34	34	35	38
2	7.0	40	0	5	11	19	27	28	32	34	36	38	39
4	6.8	40	5	23	35	37	37	37	38	38	38	40	
8	7.2	40	10	22	23	29	30	30	32	34	38	40	
18	9.8	40	34	40									
Untreated	0	40	0	0	0	2	7	10	12	19	24	25	29
		-----Successive periods of time-----											
0-1	5.3	40	0	5	7	14	17	25	29	34	35	37	38
1-3	4.9	40	2	11	16	22	25	28	33	37	38	39	39
3-5	5.0	40	2	6	8	18	23	26	27	31	33	35	36
5-9	4.6	40	0	9	12	19	27	31	33	37	39	40	
9-20	5.7	40	1	12	19	26	30	33	34	34	34	35	35
20-44	4.8	40	11	22	28	29	30	34	35	37	39	40	
Untreated	0	40	0	0	0	0	3	5	8	13	16	21	25

1/ Each weight on average of 5 samples.

Table 6.--Cumulative mortality of *D. brevicomis* confined with Jeffrey x ponderosa pine resin vapors collected at different time intervals

Time	Resin weight loss[1/]	Total beetles used	Days after start of test: 3	4	5	6	7	8	9	10	11	12	13
Hours	Milligrams	Number	Number										
			------Cumulative periods of time------										
3	4.1	40	3	3	6	11	20	25	32	35	36	38	40
6	5.1	40	1	1	5	6	16	25	28	31	32	36	37
12	5.3	40	3	5	6	10	12	15	22	30	34	36	38
24	6.8	40	3	9	16	21	26	29	33	35	38	38	39
48	7.5	40	4	10	13	17	21	27	35	37	38	39	40
Untreated	0	40	0	0	3	5	7	11	15	22	28	30	31
			------Successive periods of time------										
0-3	3.7	40	0	0	2	7	12	19	26	29	31	35	39
3-6	3.1	40	1	1	2	8	15	20	27	32	37	37	38
6-12	3.2	40	0	2	4	13	19	22	26	31	36	37	39
Untreated	0	40	0	0	5	6	9	13	16	23	32	32	34
12-24	4.0	40	1	1	4	7	12	16	21	27	29	35	36
Untreated	0	40	0	0	3	4	7	8	11	15	20	25	27
24-48	4.8	40	8	10	14	22	27	33	34	39	39	39	39
Untreated	0	40	0	1	5	7	13	18	23	27	29	32	24

1/ Each weight an average of 5 samples.

Comparison of *D. brevicomis* and *D. jeffreyi*

One of the most interesting aspects of the entire bark beetle resistance problem is the relationship of essentially two monophagous bark beetles to various pines. *D. brevicomis* is restricted chiefly to ponderosa pine, though it is sometimes found on Coulter pine. *D. jeffreyi* is essentially restricted to Jeffrey pine. Ordinarily neither beetle is found on the host tree of the other.

Previous attempts to use *D. jeffreyi* with the standard fumigation procedure met with failure. A suitable procedure for this insect was to substitute two $\frac{1}{4}$-dram shell vials for the bundles used for *D. brevicomis*. Only two of these vials could be used in each cork-stoppered 30 cc. test tube. Thus the number of replicates had to be increased.

The intent was to confine the two beetles with equal vapor concentrations of the three resins; this was not obtained. The removal of the resin samples of ponderosa, Jeffrey x ponderosa, and Jeffrey pine at 24, 3, and $2\frac{1}{2}$ hours respectively resulted in an average resin weight-loss in the fumigation chambers of 3.0, 3.5, and 5.0 milligrams respectively. The variable absorption by cork and the slight loss of vapor when the resin samples were removed were additional uncontrollable factors. Thus the vapor doses of the resins were not comparable.

There were 10 replicates of 8 beetles for *D. brevicomis* and 20 replicates of 2 beetles each for *D. jeffreyi*. The data from the test is summarized in table 7, and graphically presented in figure 7.

Discussion: The results must be viewed with caution because of the lack of equal resin weight-loss and because of the uncertain action of the cork. However, one of the most interesting aspects is the shift of Jeffrey pine curve from a position of toxicity with *D. brevicomis* to a position of innocuousness with *D. jeffreyi*. This could mean that the reaction of a beetle to resin vapors is a degree of measure of the possible beetle-host relationship. The test also illustrates the lack of toxicity of low dosages of Jeffrey pine resin to *D. brevicomis*.

Fifty-percent saturation

This test is reported though it was not altogether successful. Its purpose was to expose *D. brevicomis* to a 50 percent vapor saturation of the resin of ponderosa, Jeffrey, and Jeffrey x ponderosa pine.

The procedure was to place an excess of resin, in the resin vial, into the cork-stoppered test tube. The latter was oriented with the cork downward so that the resin vial could be removed with a minimum disturbance of the atmosphere within the test tube. The resin was allowed to vaporize for 48 hours which was a sufficient time for all three resins to reach saturation.

Table 7.--Cumulative mortality of *D. brevicomis* and *D. jeffreyi* confined with resin vapors of ponderosa, Jeffrey, and Jeffrey x ponderosa pine

Resin	Average resin weight loss	Total beetles used	Days after start of test										
			3	4	5	6	7	8	9	10	11	12	13
	Milligrams	Number	Number										
			Dendroctonus brevicomis										
Ponderosa	3.0	80	0	7	12	22	43	58	67	71	74	76	77
Jeffrey	5.0	80	3	7	15	20	31	35	45	57	64	66	68
Hybrid	3.5	80	1	3	9	12	21	31	42	51	59	66	70
Untreated	0	80	1	3	4	11	19	24	28	38	52	63	69
			Dendroctonus jeffreyi										
Ponderosa	3.8	40	5	5	13	22	29	31	33	36	38	39	40
Jeffrey	5.5	40	2	2	3	4	8	11	14	18	28	32	37
Hybrid	3.5	40	1	5	6	9	17	25	26	29	34	39	40
Untreated	0	40	2	4	5	11	17	22	27	28	30	35	38

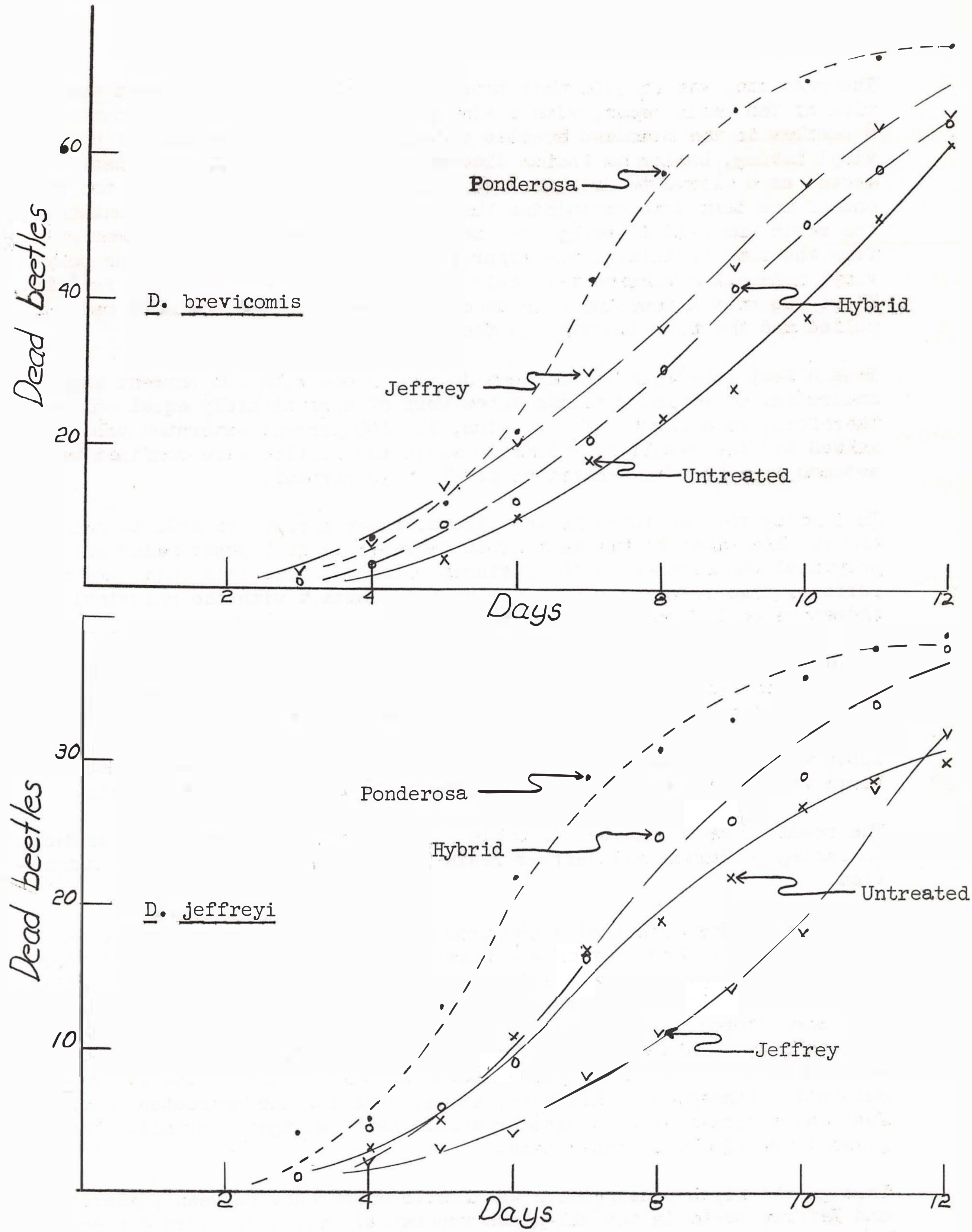

Figure 7.--Mortality rate of D. brevicomis and D. jeffreyi confined with subsaturation concentrations of ponderosa, Jeffrey, and hybrid resin.

The next step was to join this tube, presumably with 100 percent saturation of the resin vapor, with a similar one with no vapor but containing 8 beetles in the standard bundles of cells. A 2-inch section of polyvinyl tubing, having an inside diameter slightly less than the test tube, served as a sleeve to join the two tubes. It was inserted over the open end of the test tube containing the beetles. The corked tube containing the resin was held directly over the polyvinyl tube. The cork was removed from the tube containing the vapor which was then pushed into the polyvinyl sleeve. Two workers were able to make these transfers very rapidly. There was only a fraction of a second between the time the cork was pulled and the tube inserted in the sleeve.

Thus a test tube with beetles was joined to one with 100 percent vapor saturation of resin. The two tubes were of approximately equal volume; therefore, as a result of the union, the 100 percent saturated volume was halved and the resulting volume to which the beetles were confined was assumed to have a concentration of about 50 percent.

In joining the two tubes in the sleeve, every effort was made to bring the two lip edges of the test tubes together so that practically no polyvinyl was exposed to the resinous volume. Since this union was not perfect, some resinous vapors did come in contact with the polyvinyl and therefore could have been absorbed by it.

One additional factor was introduced by using the 30 cc. glass tube for the beetles. The cork which confined the resin was placed on the free end of the 30 cc. glass tube immediately after it was joined with a test tube of beetles. Half of the replicates were with 30 cc. glass tubes while the other half were with the standard 30 cc. test tubes. There were 8 beetles per replicate and 10 replicates for each resin.

The results are summarized in table 8. Figure 8 is a graphic presentation comparing ponderosa and jeffrey resin at approximately 50 percent saturation.

Discussion: The effect of a 50 percent saturation of resin vapors was decidedly lower than that of a saturated atmosphere, assuming that there was about a 50 percent saturation in the atmosphere to which the beetles were exposed. As a rule the vapors in the test chambers which were closed with cork stoppers were more toxic than those which were glass-enclosed at both ends. This was true even for ponderosa pine where fresh corks were used instead of those which had been used to stopper the 100 percent saturation atmosphere. Likewise, it was true for the untreated checks. Just why a cork-stoppered chamber should cause a higher mortality than glass is difficult to understand.

Through the first 5 days there was little difference between ponderosa and Jeffrey resin in the all-glass apparatus. But a distinct difference between them occurred after this time. This suggests a greater toxicity of Jeffrey pine resin at 50 percent saturation.

Table 8.--Cumulative mortality of D. brevicomis confined with 50 percent vapor saturation of ponderosa, Jeffrey, and Jeffrey x ponderosa pine resin

Resin	With or without cork	Total beetles used	Days after start of test										
			3	4	5	6	7	8	9	10	11	12	13
		Number	Number										
Ponderosa	w/o	40	1	2	2	3	6	8	12	13	17	19	28
	with 1/	40	2	5	6	9	13	16	23	28	29	34	36
Jeffrey	w/o	40	2	2	4	7	9	11	23	27	33	36	37
	with	40	3	5	11	13	19	21	26	34	36	37	39
Hybrid	w/o	40	2	2	5	7	8	11	16	21	23	26	33
	with	40	2	4	7	13	18	23	26	32	34	36	37
Untreated	w/o	40	1	1	3	5	9	11	13	21	27	28	31
	with	40	0	1	4	10	11	15	19	24	31	33	35

1/ Corks were unused and not those which had been used to contain the resin.

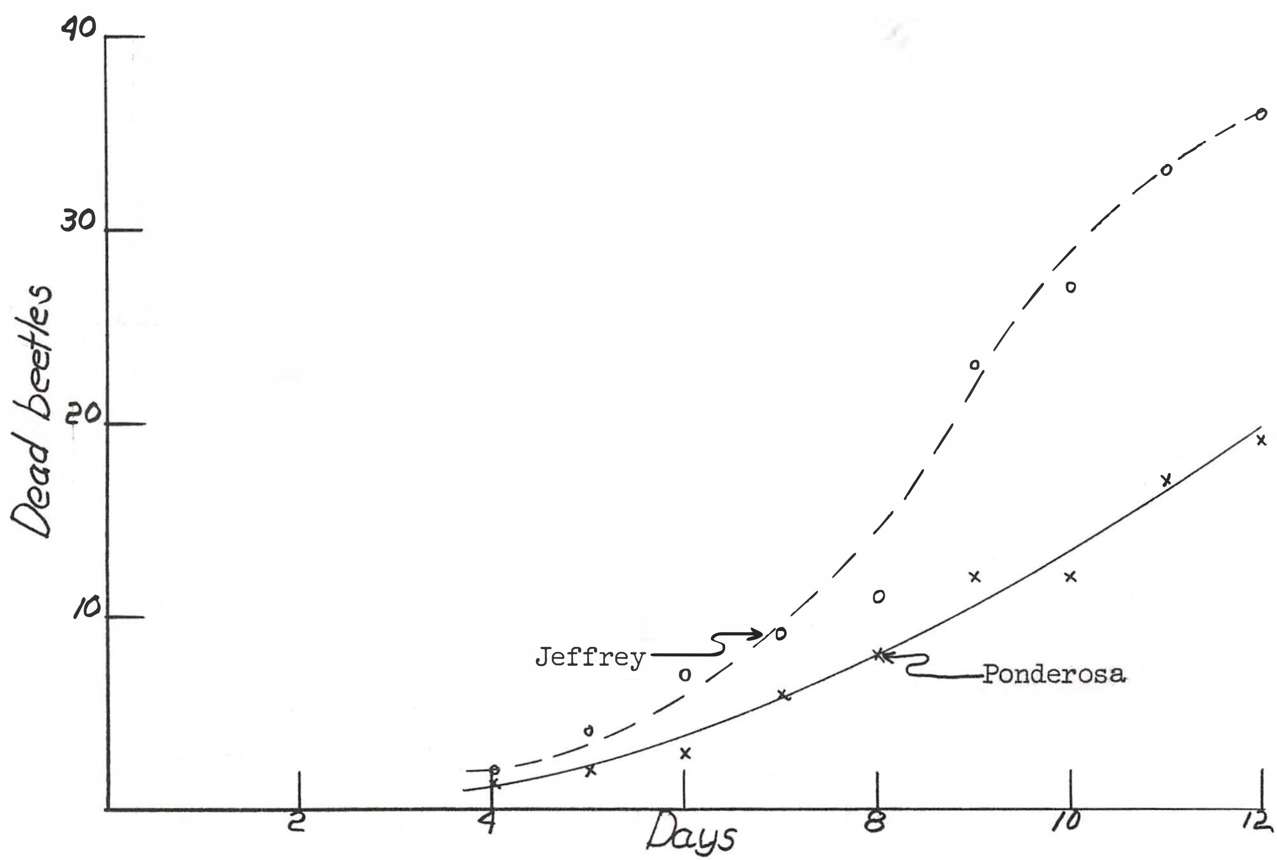

Figure 8.--Mortality rate of D. brevicomis confined with 50 percent vapor saturation of ponderosa and Jeffrey pine resin.

Resin vs. derivatives

The complex nature of resin was pointed out earlier in this report. The composition of resin, or in particular the liquid fraction, is generally determined by fractional distillation of gross resin. The fractionation involves partial vacuum and temperatures in excess of 160°C. with subsequent recovery at low temperature. Many people contend that the liquid fraction of resin and commerically prepared turpentine are synonomous. If this were the case the liquid fraction and the turpentine should have the same properties; and therefore, turpentine could be used in toxicity tests instead of the liquid fraction.

Therefore, a test was made of the fumigant property of the three materials: fresh resin, its supernatant liquid obtained by natural fractionation at room temperature, and turpentine obtained by fractionation at approximately 165°C. Standard procedures were used with the 30 cc. cork-stoppered test tube and D. brevicomis. Two bundles of 4 beetles each, thereby making 8 beetles per replicate, were first placed in the test tube. The resin was apportioned into 0.5 ml. lots but was weighed at the designated intervals in order to get the vapor dosage in the fumigation chamber.

The resin and resin product samples of ponderosa pine which were placed just inside the cork stoppers were removed from 1/2 of the replicates at 24 hours and the remaining half were removed at 48 hours. The resinous atmosphere was disturbed only slightly in removing the resin sample. The beetles were kept in the resulting resinous atmosphere for the duration of the test. The results are summarized in table 9 while figure 9 is a graphic presentation of the same data.

Table 9.--Cumulative mortaility of D. brevicomis confined with the vapors of ponderosa pine resin and its derivatives

Material	Exposure	Weight loss 1/	Total beetles used	Days after start of test: 1	2	3	4	5	6	7	8	9	10
	Hours	Milligrams	Number	Number									
Resin	24	2.5	40	0	2	4	5	6	10	21	28	30	35
	48	2.9	40	0	3	5	8	15	21	26	31	35	37
Supernatant liquid	24	2.6	40	0	0	2	3	6	8	18	27	32	37
	48	3.7	40	0	2	3	7	11	17	21	27	33	38
Turpentine	24	6.2	40	2	3	8	14	20	33	37	39	39	40
	48	7.7	40	-	11	22	27	34	38	38	39	40	40
Untreated	24	0	40	0	0	2	3	3	5	9	11	14	20
	48	0	40	0	0	0	1	3	7	11	13	16	16

1/ Each an average of 5 samples, no correction for absorption by cork

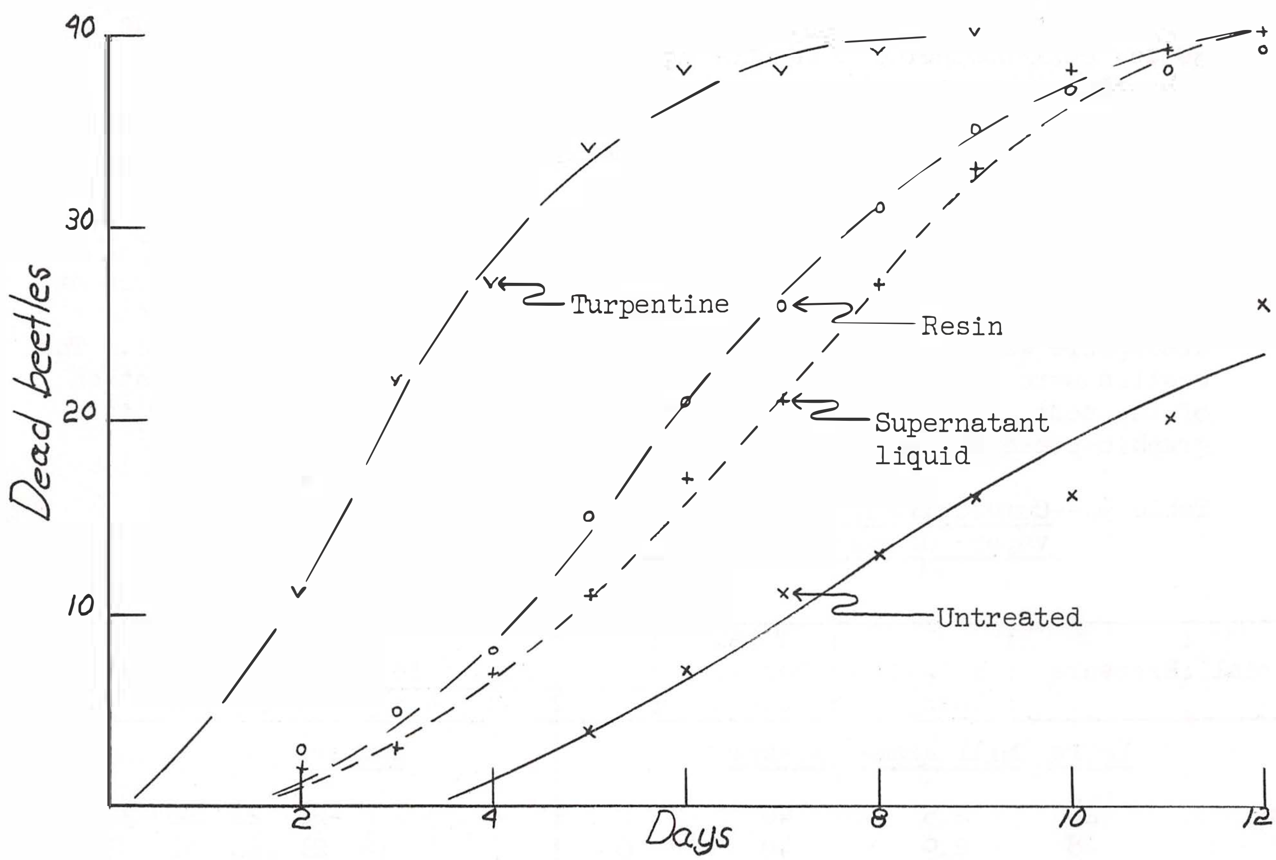

Figure 9.--Mortality rate of D. brevicomis confined with saturated vapor of ponderosa pine resin, supernatant liquid and turpentine.

Discussion: It is quite apparent that, at least in respect to vapor saturation and effect on the beetle, turpentine and the supernatant liquid of ponderosa pine resin are not synonomous. On the other hand fresh resin and supernatant liquid are almost identical in these respects. This would lead one to assume that some change was made in the volatile portion of resin when it is fractionated at 165°C. Therefore in studying toxicity continued use should be made of resin and not its commercial derivatives.

Humidity

To date all the fumigant toxicity tests have been with an atmosphere of about 70 percent relative humidity. For comparative purposes this is acceptable but for realistic purposes it is not. Moisture conditions which exist in a bark beetle gallery very likely approach 100 percent relative humidity. Thus a test was set up to compare the effects of relative humidity on fumigant toxicity in the standard test tube.

A relative humidity of about 70 percent was obtained by using the standard procedure. A 100 percent humidity was obtained by placing in the fumigation chamber a small vial of water with a cotton wad to act as a wick and an evaporating surface.

A saturated atmosphere of ponderosa and Jeffrey pine resinous vapors was obtained by a 0.5 ml. volume of fresh resin. Appropriate weighings were made to determine the loss of weight of the resin. Ponderosa resin was contained in the chamber for 48 hours before removal, while Jeffrey resin was held for only 2½ hours. The objective was to secure equal losses of weight of the resins; however, this was not accomplished. The resin samples were removed from the chamber and the chamber was restoppered with as little disturbance as possible to the contained volume.

Five replicates of 8 beetles each were used for each combination of resin and humidity in this test. The results are summarized in table 10 while figures 10, 11, 12, and 13 present a graphical interpretation of some of the more interesting aspects of these results.

Table 10.--Cumulative mortality of *D. brevicomis* confined at different humidities with the resinous vapors of ponderosa and Jeffrey pine

Humidity	Resin	Resin weight loss	Total beetles used	Days after start of test: 1	2	3	4	5	6	7	9	11	13
Percent		Milligrams	Number	Number									
70	Ponderosa	4.2	40	0	1	1	3	11	15	24	37	40	
	Jeffrey	8.8	40	1	17	27	34	35	38	40			
	Untreated	0	40	0	2	2	2	4	6	7	12	22	27
100	Ponderosa	4.6	40	0	0	1	1	2	2	2	9	12	20
	Jeffrey	7.1	40	6	33	38	39	39	40	40			
	Untreated	0	40	0	0	0	0	0	0	1	3	10	18

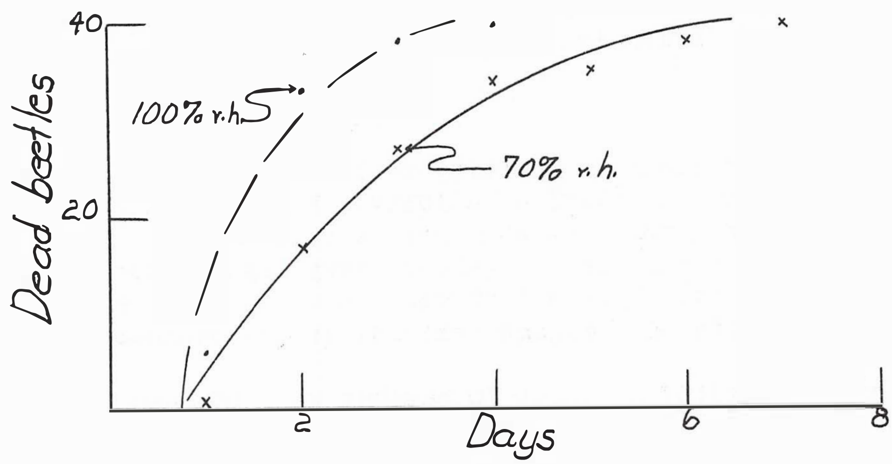

Figure 10.--Mortality rate of D. brevicomis confined with saturated vapor of Jeffrey pine resin at 70 percent and 100 percent relative humidity.

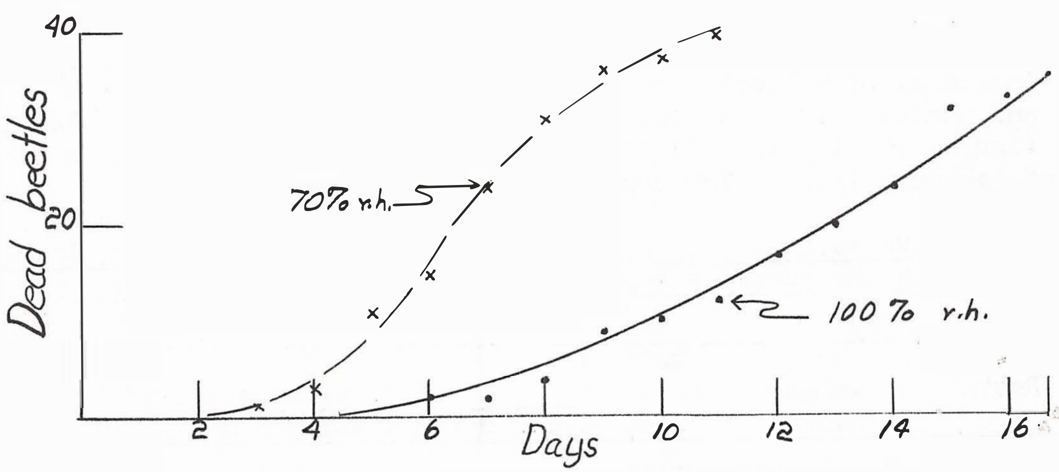

Figure 11.--Mortality rate of D. brevicomis confined with saturated vapor of ponderosa pine resin at 70 percent and 100 percent relative humidity.

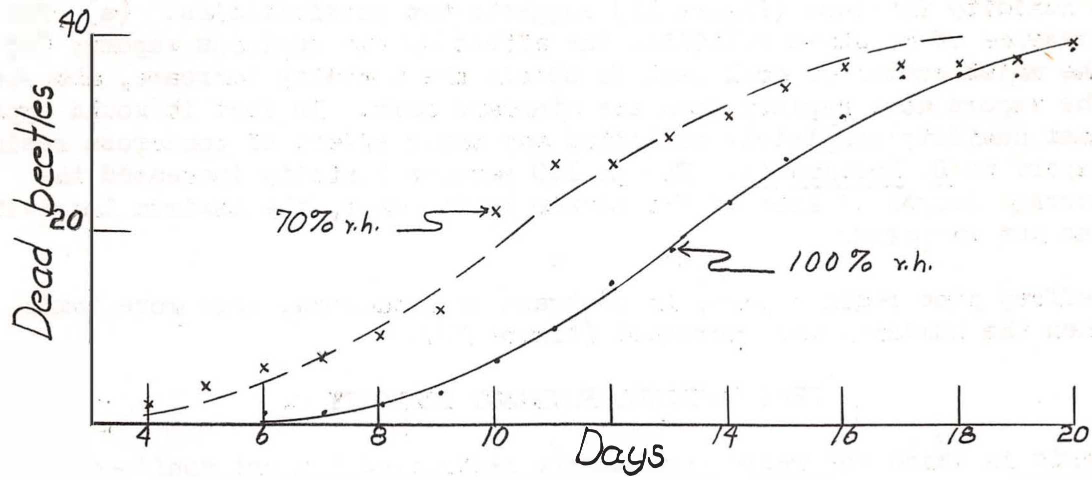

Figure 12.--Natural mortality rate of D. brevicomis confined with atmospheres of 70 percent and 100 percent relative humidity.

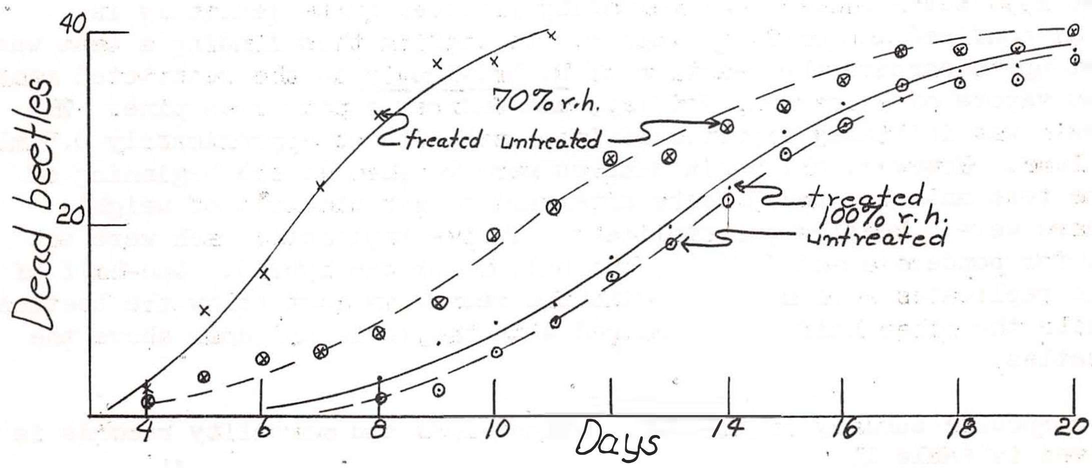

Figure 13.--Mortality rate of D. brevicomis as affected by the relative humidity of confined, treated and untreated atmosphere--treated was vapor saturation of ponderosa pine resin.

Discussion: The results, which are so extreme and which follow no consistent pattern, are difficult to interpret, but they suggest that humidity can play an important role in fumigant toxicity.

The great difference in the effect of ponderosa resin with and without a humidity increase (figure 11) suggests two possibilities. (a) The presence of moisture nullified the effect of the resinous vapors; (b) the moist cotton or cork used to obtain the humidity increase, absorbed the vapors more rapidly than the standard cork. In fact it would appear that humidity completely nullified any toxic effect of ponderosa resinous vapors to D. brevicomis. Though 100 percent humidity increased the average length of life of the beetle by two days, the maximum longevity was not increased.

Jeffrey pine resin vapors, in contrast to ponderosa, were more toxic when the humidity was increased (figure 10).

SEMI-CONFINED FUMIGANT TOXICITY

Tests in which the resin vapors were restricted but not confined in the fumigation chamber are termed semi-confined fumigant toxicity tests in this report. This condition was obtained by using a section of glass tubing with a 30 cc. volume. A fumigation chamber of this type permitted the containment of the bundles of beetles in restricted area and yet permitted free passage of the resinous vapors by stoppering only one of the openings. By proper combinations in the placement of cork and resin, the resin vapors could be made to move upward or downward over the restricted beetles.

The 1956 tests showed that mortality differed quite radically in semi-confined and confined vapors. To confirm this finding a test was set up to compare the reaction of D. brevicomis to the restricted resinous vapors of ponderosa, Jeffrey, and Jeffrey x ponderosa pine. The resin was initially apportioned into each tube at approximately 0.5 ml. volume. However, all resin samples were weighed at the beginning of the test and at 2 and 3 weeks afterward to get the loss of weight. There were 8 beetles per replicate. Twelve replicates each were set up for ponderosa and Jeffrey, but only 8 for the hybrid. One-half of the replicates were arranged with the resin and cork below the beetles while the other half were arranged with the resin and cork above the beetles.

A composite summary of the resin weight-loss and mortality records is given in table 11.

Discussion: The results are similar to the 1956 results and again present a paradox. The ponderosa resin vapors caused the most rapid mortality while Jeffrey resin caused the least rapid. The hybrid was intermediate. The position of cork and resin had no appreciable effect on the rate of mortality despite the fact that this placement did affect the amount of resin which vaporized. The difference in resin weight loss was greatest with ponderosa resin, least with Jeffrey, and intermediate with the hybrid. These weights show that all the volatile fraction had not vaporized at 14 days.

Table 11.--Cumulative mortality of D. brevicomis in restricted resin vapors of ponderosa, Jeffrey and Jeffrey x ponderosa pine

Resin	Placement of cork and resin	Av. resin wt.-loss 14-days	Total beetles used	Days after start of test									
				2	3	4	5	6	7	8	9	10	11
		Milligrams	Number	Percent									
Ponderosa	Below	90.7	48	2	6	10	14	31	54	72	81	89	100
	Above	45.0	48	0	2	4	12	25	37	70	77	89	100
Jeffrey	Below	59.4	48	2	2	2	2	2	10	18	35	43	56
	Above	66.4	48	0	0	0	2	8	10	18	33	41	56
Hybrid	Below	70.4	32	0	0	0	3	6	13	31	47	69	75
	Above	57.1	32	0	0	3	6	9	25	56	72	88	94
Untreated	Below	-	48	0	0	0	2	12	14	20	27	54	79
	Above	-	48	0	2	4	6	10	14	20	35	45	66

One possible explanation for the lack of apparent toxicity of Jeffrey resin is its mild paralytic effect. D. brevicomis always shows a partial paralysis to Jeffrey resin vapor. Under the conditions of this test the paralysis might have been too slight to cause mortality but just enough to reduce the beetle's activity and thereby extend its natural life. It is assumed, in this case, that a non-feeding beetle has a certain amount of stored food which enables it to do a given amount of work. If such a beetle has its activity partially curtailed, it is conceivable that its life span without food could be increased.

This same reasoning might be used to explain the more rapid rate of mortality with ponderosa resin vapors. Though it is not possible to detect any "stimulating" effect of ponderosa resin on D. brevicomis, such an effect could exist, and therefore, in the absence of food cause a more rapid rate of mortality. Though a partial paralysis was noted in the presence of Jeffrey resin, no "stimulating" effect was observed in the presence of ponderosa resin.

CONTACT TOXICITY

The work on contact toxicity in 1956 was rather meager and the procedure which was used was rather limited in its usefulness. In 1957 it was therefore the decision to continue with small "procedure-exploring" tests while continuing the more extensive tests on fumigant toxicity. Three of these exploratory tests were carried out. The samples were small and the conditions not too rigid. However, the results of the

tests did indicate the following two points. (1) A single coating of fresh resin of ponderosa, Jeffrey, or Jeffrey x ponderosa pine did not appreciably hasten the death of D. brevicomis or D. jeffreyi. (2) The physical properties of the resin could play a major role in the effect of resin on the beetle.

A more extensive and closely controlled experiment was designed to determine the effect of resin as measured by mortality as well as by the feeding behavior and ability of the beetle. The basic treatment was a complete coating of gross, fresh, uncrystallized resin. This was obtained by very quickly submerging a beetle in the resin. After this almost instantaneous immersion, the beetle was placed on blotting paper for a minute or two so that all excess resin was removed. Thus the beetle was left with a complete coating of resin. One-half of these treated beetles were then placed individually in $\frac{1}{4}$-dram vials for observations on mortality rate. The other half were subjected to tests to study their feeding behavior and capabilities. This was accomplished by placing them individually in a 4-inch length of glass tubing, 5 mm. i.d., which was plugged with a 2-inch length of ponderosa pine middle bark (the spongy, moist bark between the phloem and the outer dry bark). This tube was plugged at each end with a small piece of lumite mesh screening and was placed at an angle of about 10° with the beetle at the lower end.

The test was maintained at 70°F. without light. Sixty beetles were treated with each of the three resins: ponderosa, Jeffrey, and Jeffrey x ponderosa. Daily observations were made on mortality and on the amount of feeding by the beetles in the tubes with bark. Each 2-inch bark plug was given a value of 4 feeding units. The amount of feeding by a beetle was measured by the number of units it had traversed. Thus, a beetle which chewed through the entire 2-inch length had consumed 4 units. The results of the mortality observations are given in table 12 while table 13 gives the results of the observations of the feeding and behavior and capabilities. Figure 14 compares the mortality of treated and untreated beetles and figure 15 compares the mortality of feeding and non-feeding untreated beetles.

Discussion: The results of the experiment were quite unusual in some respects. There was no difference between the 3 different resins as expressed in the mortality rate of the beetles (figure 14). But generally there was a distinct difference between treated and untreated beetles. It should be emphasized that the conditions of the test were quite unlike those which the beetle encounters in a living tree, though they may not be too different from those found in a cut log.

Feeding did not appear to have lengthened the life of the beetle. In fact it looks as if it may have shortened it a bit.

That part of the test in which beetles were treated and then allowed to feed produced rather unexpected results. Beetles treated with ponderosa resin were either more inclined or more able to feed than untreated beetles. Likewise those treated beetles which did feed were as capable in the amount of feeding as untreated beetles. In these results there is a

suggestion of a relationship between resin and beetle other than a toxic or deleterious one.

Table 12.--Cumulative mortality of D. brevicomis with a single coating of resin

Resin	Test container	Total beetles used	Days after start of test									
			2	3	4	5	6	7	8	9	10	11
		Number	Number									
Ponderosa	Vial	30	1	2	5	7	10	12	16	20	20	27
	Tube	25	0	0	2	10	15	21	22	22	24	24
Jeffrey	Vial	30	2	2	6	8	10	13	17	19	26	28
	Tube	30	5	9	10	13	16	19	25	26	30	30
Hybrid	Vial	30	1	1	5	7	9	13	18	20	26	27
	Tube	26	1	2	4	9	12	18	21	22	25	26
Untreated	Vial	30	0	0	1	2	4	7	9	11	24	27
	Tube	28	0	0	1	3	6	8	14	19	22	25

Table 13.--Effect of a single coating of resin on the feeding behavior and capability of D. brevicomis

Resin	Total beetles used	Days after treatment					
		1	2	3	4	5	6
		Feeding beetles					
	Number	Number					
Ponderosa	30	22	25	26	26	26	
Jeffrey	30	1	4	7	11	13	
Hybrid	30	1	7	11	18	18	
Untreated	30	2	3	3	6	6	
		Amount of feeding					
	Number	Units 1/					
Ponderosa	30	27	48	72	79	81	81
Jeffrey	30	1	4	11	20	24	28
Hybrid	30	1	7	26	37	39	41
Untreated	30	3	6	9	16	18	18
		Feeding per feeding beetle					
	Number (3/)	Units 2/					
Ponderosa	--	1.2	1.9	2.8	3.0	3.1	3.1
Jeffrey	--	1.0	1.0	1.6	1.8	1.8	2.1
Hybrid	--	1.0	1.0	1.6	2.1	2.2	2.2
Untreated	--	1.5	2.0	3.0	2.7	3.0	3.0

1/ 120 units maximum for each resin.

2/ 4.0 units maximum for each resin.

3/ Varies each day; same as figures for number of feeding beetles.

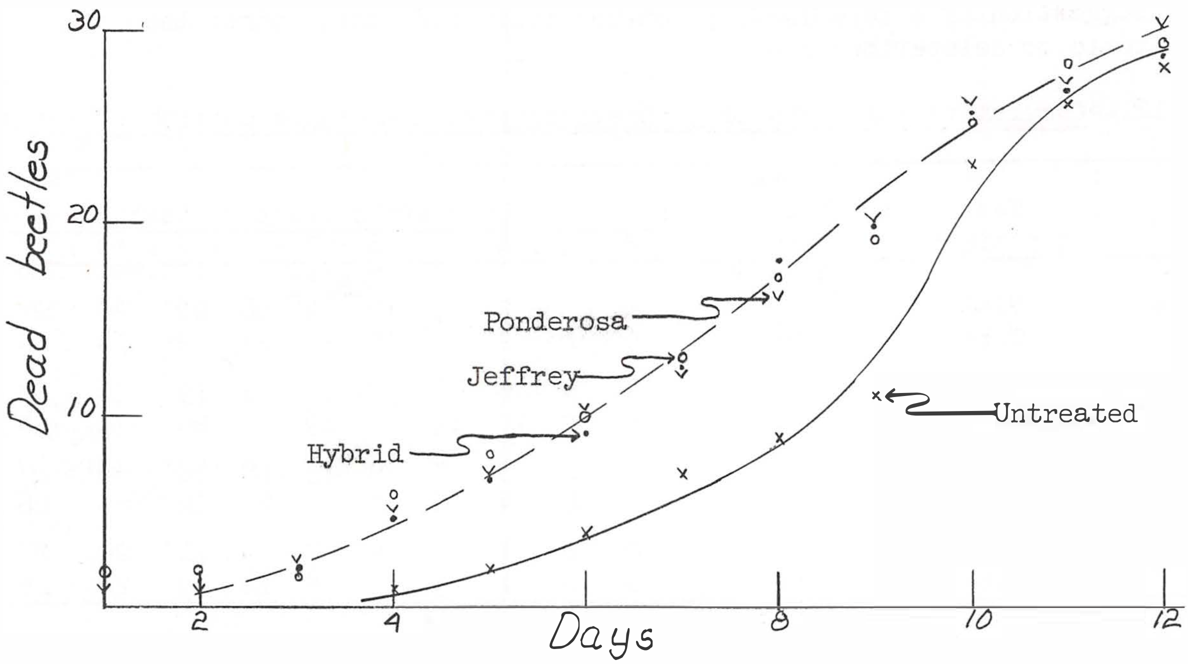

Figure 14.--Mortality rate of D. brevicomis after brief contact with ponderosa, Jeffrey, and hybrid pine resin.

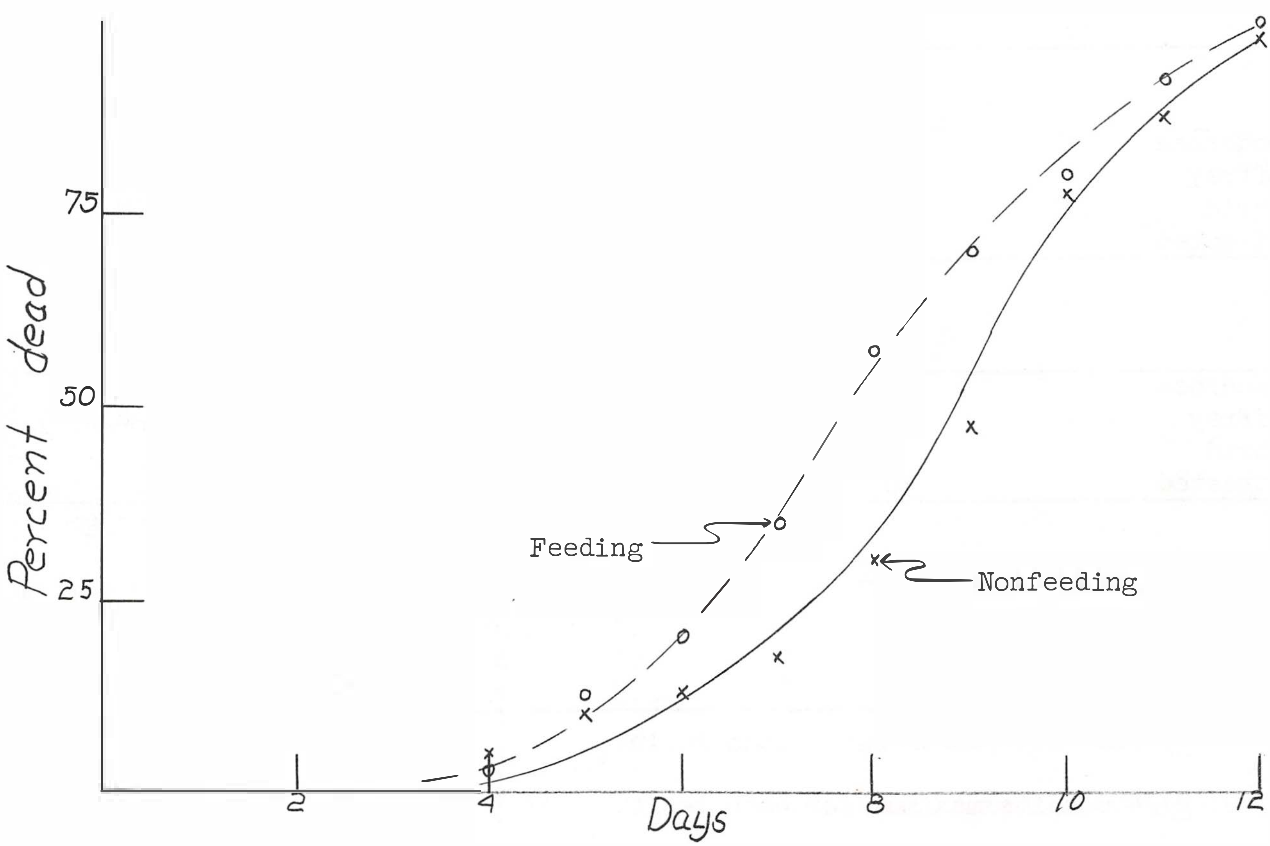

Figure 15.--Mortality rate of D. brevicomis affected by feeding on ponderosa pine middle bark.

RESPONSE

The second major division of the overall problem of host resistance is bark beetle response. Expressed slightly differently, to what stimulus, if any, does a bark beetle respond in order to reach a tree? This is a very vital, but none-the-less perplexing, question.

There are two theories to explain a bark beetle's reaching a tree. The first one (Person[4]) states in essence that a bark beetle (in this case specifically western pine beetle) is attracted to certain trees or certain conditions of a tree. That is, the insect uses its olfactory sense. The second one (Callaham[5]) states in essence that beetles fly at random through the forest. They bore into a pine tree and attempt to establish an attack. If successful (and their success rests largely on the quality and quantity of resin flow) the tree is made more attractive to other beetles in the area who then are attracted to the tree in large numbers. Throughout the literature and in unpublished reports about the apparent "attractiveness" of certain trees the weight of circumstantial evidence, especially with western pine beetle, is quite impressive. The fact remains that no conclusive evidence has been offered to substantiate any theory.

At the outset response was recognized as being equally important as the toxicity studies. However the initial emphasis on the project was toward toxicity studies, first, because the previous work on the project was in this direction and, second, because suitable techniques for response studies seemed to present even greater problems than those for toxicity. Nevertheless some probings were made into the matter of procedures.

The first requisite for response studies is a device for measuring the response of a beetle. The usual assumption has been that bark beetles orient themselves toward their host largely through the sense of olfaction, therefore the measuring device has been called an olfactometer.

Two such devices have been used for measuring the response of bark beetles.[6] [7] They were quite similar in design but quite different in

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4/ Person, H.L. 1928. Tree selection by the western pine beetle. Jour. Forestry 26:564-578, illus.

5/ Callaham, R.Z. 1952. Host selection and host susceptibility in the western pine beetle-ponderosa pine complex. B.E.& P.Q. Forest Insect Lab. Berkeley, Calif. 8 pp.

6/ Gordon, Aaron, 1932. Tree injection experiments in white fir and ecological studies of insects attacking yellow pine---. B. E. & P. Q. Forest Insect Lab. Berkeley, Calif. 10 pp., illus.

7/ Callaham, R.Z. 1952. Studies of the olfactory and air current tropisms of the western pine beetle. B.E. & P.Q. Forest Insect Lab. Berkeley, Calif. 10 pp., illus.

proportion. The basic design involved the use of two tubular arms held in a horizontal position and joined at one end so that each projected horizontally at 180° to the other. Where the two arms were joined, arrangements were made for release of the beetles. Air was drawn over a source substance at the end of each arm so that it passed over the beetles, and was exhausted below the beetles. Each tube had a light source at the distal end. In Gordon's apparatus the arms were about 1 foot long and about $1\frac{1}{2}$ inches in diameter, while in Callaham's the arms were 12 feet long and 22 inches in diameter.

A device was constructed which differed from these two (figure 16) in that the two arms were at an angle of about 45°. This is in some respects quite similar to several Y-type olfactometers used with other insects. The entire device was of plexiglass with a strip of lumite mesh screening serving as a walk-way for the beetles.

The general procedure was to place the olfactometer with about a 10° incline from "g" to "c" while "c" to "c'" was level. The beetles were placed at "g" with a strong light source at "c" "c'". Air was pulled through the apparatus from "a" "a'" to "g" by a blower fan intake connected at "g".

Exploratory tests were made to determine the suitability of this apparatus. The first work was with *Dendroctonus valens* Lec. The beetles had been collected about three months before the tests and had been held in refrigeration at 35°F. The first test was to determine the rate of selection between the two arms when there was no material at the source of the arms. Five small lots of beetles were used. They showed nearly an equal choice for the two arms with 22 going to the left and 23 to the right. These same beetles were run through again keeping them separate according to their original direction of choice. Most of these were rerun a second time, thus the discrepancy between the numbers of initial and rerun beetles. The following is a listing of the results of the second and third runs.

Left - initial choice 22		Right - initial choice 23	
Rerun choice		Rerun choice	
Left	Right	Left	Right
3	1	1	4
3	0	2	2
4	0	0	3
6	2	3	6
3	3	2	4
3	1	1	3
5	0	1	6
3	3	0	5
30	10	10	33

After this, the materials in the arms were made dissimilar by placing at the origin of one arm a small bead-sized piece of *D. valens* pitch

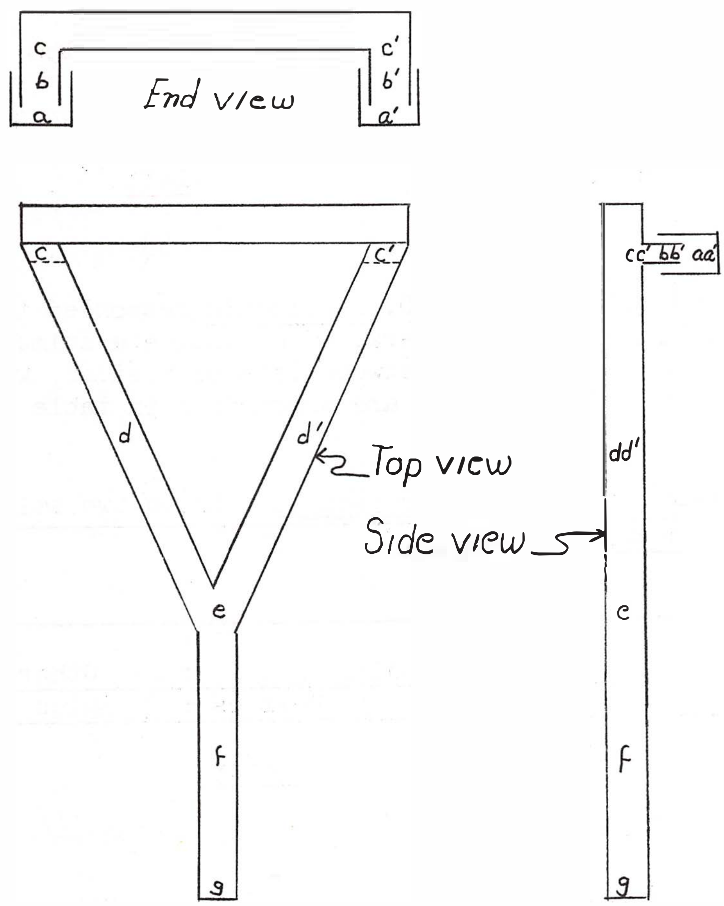

Figure 16.--Diagrammatic views of olfactometer, scale about 1/4 actual size, all material plexiglass.

tube from Monterey pine. The particle was shifted from one arm to the other between runs. The overall results of 13 different small lots of beetles showed 58 going to the pitch tube particle and 31 going to the arm with nothing.

The work then turned to D. brevicomis. For both the initial and rerun choice, this species was quite similar to D. valens. Here too some beetles were re-run twice to cause differences in number of beetles used. The following lists the results of 19 initial runs and 10 reruns.

Left initial choice		Right initial choice	
153		156	
Rerun choice		Rerun choice	
Left	Right	Left	Right
103	39	53	106

An attempt was then made to see if D. brevicomis responded to possible stimuli such as phloem and middle bark. The candidate stimulus was changed from one arm to the other between lots of beetles, which numbered from 15 to 20 per lot. The results are summarized in table 14.

Table 14.--Selection by D. brevicomis between two stimuli in an olfactometer

Test	Lots of beetles used	One choice		Other choice	
		Stimulus	Beetles	Stimulus	Beetles
Number	Number		Number		Number
1	12	1-day old phloem	74	Check 1/	85
2	6	2-day old phloem	42	Check	51
3	3	Middle bark	20	Check	20
4	2	Middle bark	14	Phloem	14

1/ Check was an untreated stream of air equal in speed and temperature to the opposite arm.

Discussion: The early work with D. valens showed some promise for the apparatus. However it also showed that 50 or more beetles would have to be used to offset the tendency for beetles to go left or right. It also

indicated that response might be measured by the apparatus. The work with *D. brevicomis* showed that either the apparatus was unsuitable or the stimuli were not the proper ones. Changes will have to be made before the apparatus is suitable for use with *D. brevicomis*.